Report

Expression of BP1, a novel homeobox gene, correlates with breast cancer progression and invasion[★]

Yan-gao Man[1], Sidney W. Fu[2], Arnold Schwartz[3], Joseph J. Pinzone[2,4], Samuel J. Simmens[5], and Patricia E. Berg[2]

[1]*Department of Gynecologic and Breast Pathology, Armed Forces Institute of Pathology;* [2]*Departments of Biochemistry and Molecular Biology;* [3]*Department of Pathology;* [4]*Department of Medicine;* [5]*Department of Epidemiology and Biostatistics, The George Washington University Medical Center, Washington DC, USA*

Key words: BP1, breast cancer, ductal carcinoma *in situ*, homeobox, invasion, progression

Summary

Background: Our previous studies revealed that the mRNA encoded by BP1, a member of the homeobox gene superfamily of transcription factors, was expressed in leukemia and infiltrating breast ductal carcinoma (IDC). This study investigated the immunohistochemical profile of BP1, to determine whether the expression of BP1 protein correlated with breast tumor progression and invasion and whether BP1 was co-localized with erbB2.

Design: Paraffin sections from normal reduction mammoplasties ($n = 34$) and a variety of *in situ* and invasive breast cancers ($n = 270$) were either singly immunostained for BP1, or doubly immunostained for BP1 plus either erbB2 or Ki-67.

Results: The prevalence of BP1 positive cells and the intensity of BP1 immunoreactivity increased with the extent of ductal proliferation and carcinogenesis. BP1 expression was barely detectable in normal reduction mammoplasties compared to distinct staining in 21, 46, and 81% of hyperplastic, *in situ*, and infiltrating lesions, respectively. In cases with co-existing normal, hyperplastic, *in situ*, and invasive lesions, the tumor cells of the invasive lesions consistently showed the highest frequency and the highest intensity of BP1 immunostaining, followed by *in situ* tumor cells. Double immunostaining revealed that BP1 co-localized with a subset of erbB2 positive cells in all 15 *in situ* and IDC tumors examined, and that BP1 positive cells had a substantially higher proliferation rate than morphologically similar cells without BP1 expression.

Conclusion: These findings suggest that BP1 is an important upstream factor in an oncogenic pathway, and that expression of BP1 may reliably reflect or directly contribute to tumor progression and/or invasion.

Introduction

Homeobox genes encode transcription factors that are essential for early development. Increasing evidence points to a role for homeobox genes in breast cancer (reviewed in [1]). A recent paper examined the expression of the large homeobox gene family called HOX in normal and malignant breast tissues, finding that some HOX genes were activated in tumor tissue, some repressed and others unaffected [2]. Studies of specific HOX genes are in agreement with this. For example, enforced HOXB7 expression in SkBr3 breast cancer cells induced bFGF expression and increased the growth rate, serum-independent growth and the ability of cells to form colonies in semisolid media [3]. Nude mice injected with SkBr3/

HOXB7 cells developed tumors [4]. Importantly, repression of HOX A5 in breast cancer resulted in the loss of p53 expression [5].

BP1, a gene cloned in our laboratory, is a member of the distal-less (DLX) family of homeobox genes [6]. BP1 is normally expressed during early hematopoiesis in CD34 negative cells, during erythropoiesis in fetal liver, and in placenta and kidney [6, 7]. Our recent studies revealed that BP1 mRNA was detectable in 80% of infiltrating breast ductal carinoma (IDC), whereas it was undetectable in eight of nine matched normal controls, as measured by RT-PCR ([8] unpublished data), making it the first DLX gene to be strongly implicated in breast cancer. These studies also showed that BP1 expression was significantly higher in estrogen receptor (ER) negative than in ER positive breast tumors, 100 versus 73% ($p = 0.03$), and in the tumors of African-American women than in Caucasian women, 89 versus 57% ($p = 0.04$). The exclusive or preferential expression of BP1 in IDC, in ER

negative tumors, and in African-American women, if confirmed on a larger scale, could have a number of scientific and clinical implications. First, as has been well documented, ER negative tumors are clinically more aggressive and have a worse prognosis compared to ER positive tumors, suggesting that the expression of BP1 may reliably reflect or directly contribute to the aggressiveness of breast tumors [9, 10]. Second, our previous studies in leukemia have revealed that BP1 has oncogenic properties, including modulation of cell survival ([7]; unpublished data), suggesting that the high percentage (80%) of BP1 expression in IDC might enhance breast tumor growth rate and invasion. Based on these observations, the goal of this study was to elucidate the immunohistochemical profile of BP1 expression in order to determine whether the expression of BP1 protein correlates with breast tumor progression and/or invasion, and whether BP1 positive cells have a more aggressive biologic phenotype. Moreover, this study attempted to assess whether BP1 co-localizes with erbB2, a well-defined onco-protein that maps near BP1 on chromosome 17 [11, 12].

Materials and methods

Immunohistochemistry

Formalin-fixed, paraffin-embedded human breast tissues from reduction mammoplasties ($n = 34$) of individuals with no family history of breast cancer, no mammographic and histological breast abnormalities, and from patients with a wide variety of *in situ* and invasive breast lesions ($n = 270$) were retrieved from the files of The Armed Forces Institute of Pathology. Consecutive sections at 4–5 μm thickness were made and placed on positively charged microscopic slides. The first and last sections from each case were stained with hematoxylin and eosin for morphological classification, based on our published criteria [13].

A polyclonal antibody against a BP1 peptide was made from rabbit, as described [6]. A monoclonal anti-human Ki-67 antigen (clone MIB-1) was purchased from DAKO (Carpinteria, CA). A monoclonal anti-human erbB2 antigen (clone NCL-CBE-356) was purchased from Vector (Burlingame, CA, USA). Immunohistochemical staining was carried out using the ABC method, as previously described [14, 15].

Before application to experimental cases, we used several methods to verify BP1 antibody specificity and sensitivity. First, the BP1 antibody was pre-absorbed with the BP1 peptide at different concentrations before incubation with sections. Second, BP1 antibody was diluted to different concentrations and incubated with consecutive sections from multiple types of breast tumors, and from tissue culture cells with different levels of BP1 expression. Third, the primary antibody was substituted with PBS or normal serum, or the secondary antibody was omitted from the immunostaining

sequence. The pre-absorption of the primary antibody, the substitution of the primary antibody with PBS or normal serum, and the omission of the secondary antibody resulted in total negativity in all the sections tested.

To assess the potential co-localization of BP1 and erbB2, consecutive sections from 15 previously identified erbB2 positive tumors were singly or doubly immunostained for these two molecules using our published protocols [14, 15]. Cells with staining of +3 were scored as erbB2 positive [16, 17]. To monitor BP1 expression associated with cell proliferation, sections from morphologically similar *in situ* and invasive tumors with ($n = 10$) and without ($n = 10$) BP1 expression were double immunostained for Ki-67 and BP1. The cell proliferation rate in 3–5 morphologically similar ducts from each case was calculated, and the rates among cases in each category were averaged and statistically compared.

The expression status of ducts and acini lined by ≥ 40 epithelial cells was independently examined by two investigators (Y-G.M. and A.M.S.) with a >95% concordance. One investigator (A.M.S.) reviewed the samples in a blinded fashion. A given cell was considered BP1 positive if distinct chromogen coloration was consistently seen in its cytoplasm or nucleus in at least two duplicates of the same immunostaining procedure and > 5% of its entire cell population showed distinct BP1 immunoreactivity.

Statistics

Group differences were tested by the Pearson χ^2 test. The *p*-values obtained using this procedure, are approximate for Table 2–4 due to the lack of independence among the samples.

Results

BP1 positivity increases with ductal proliferative and neoplastic state

To assess the possibility that the level of BP1 expression might increase with ductal proliferative and neoplastic states, several approaches were used. First, the percentage of BP1 positive cells was assessed in the following breast tissue samples: normal ($n = 34$), hyperplasia ($n = 70$), *in situ* carcinoma ($n = 100$) and invasive carcinoma ($n = 100$), and values were statistically compared.

Among normal breast tissue samples, 30 (88.2%) were completely devoid of BP1 immunostaining, and 4 (11.8%) contained BP1 positive cell clusters, which accounted for less than 5% of the total cell population; the immunostaining did not meet the criteria for BP1 positivity (Table 1 and Figure (1A). As the histopathologic lesions progressed through ductal hyperplasia, atypical hyperplasia, carcinoma *in situ* and invasive carcinoma, the fractional cellular immunoreactivity of

the lesional ductal component increased ($p < 0.0001$). Among 70 hyperplastic ductal lesions, 15 (21.4%) were BP1 positive (Figure (1B)–(D)). Forty-six of the 100 (46%) *in situ* breast tumors, including cribriform and papillary subtypes, were BP1 positive (Figure (1E)–(F)), while 81 of the 100 (81%) of invasive tumors, including ductal ($n = 91$) and lobular ($n = 9$) types were BP1 positive (Figure (1F)–(G)). No significant difference in BP1 immunostaining was observed between the ductal and lobular lesions (data not shown).

There was also a substantial increase in the number of BP1 positive cells and intensity of BP1 immunostaining as histological entity progressed from non-invasive to invasive (IDC > DCIS > hyperplasia > normal) ($p < 0.0001$). Of 193 ducts analyzed from normal reduction mammoplasties, 17 (8.9%) were BP1 positive (Table 2). In hyperplasia, 67 of 374 ducts (17.9%) were BP1 positive; in those cases, BP1 positive

Table 1. Percentages of BP1 (+) cases among normal and different breast lesions

Lesion	Number of cases	BP1 positive (%)
Normal	34	0*
Hyperplasia	70	15 (21.4%)
In situ	100	46 (46.0%)
Invasive	100	81 (81.0%)

Histological type differences significant at $p < 0.0001$.
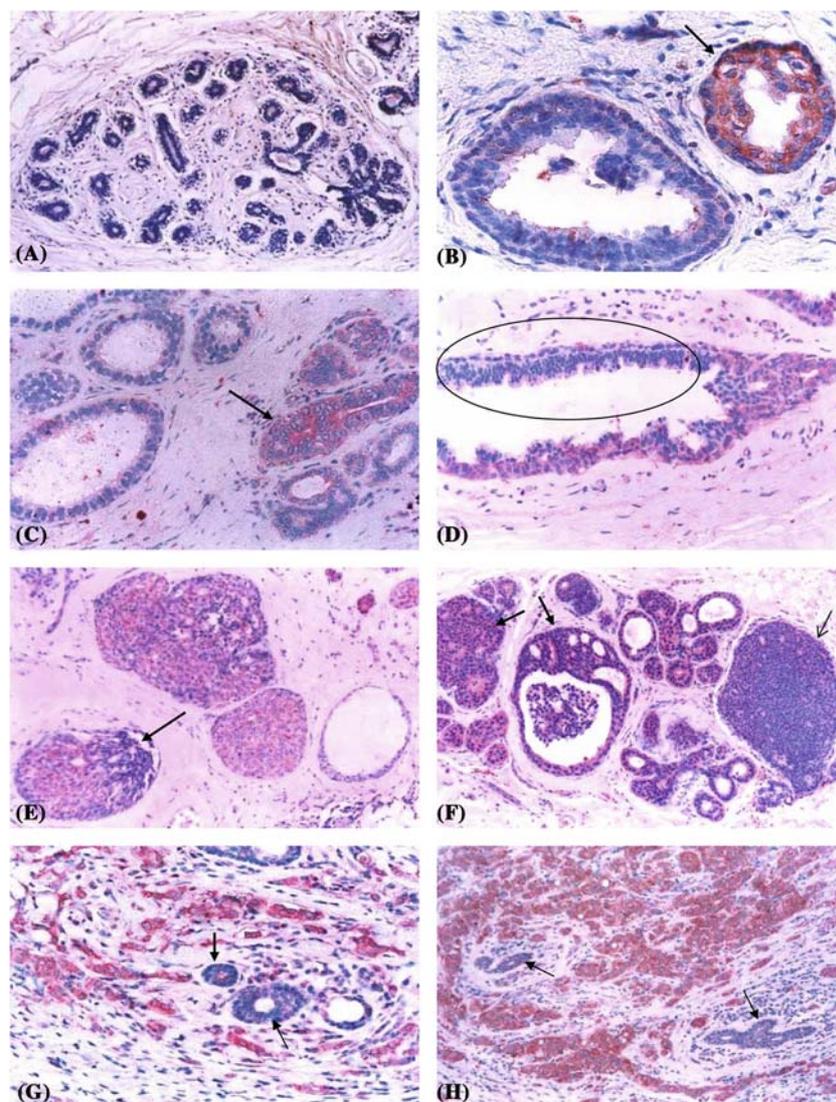*4 (11.8%) contain BP1 (+) cell clusters, but only in 1–3% of the total cells.

Figure 1. Expression status of BP1 in normal and different breast lesions. (A) Normal reduction mammoplasty. 100×. (B)–(D) Intraductal hyperplasia (IDH). In (B) and (C) arrows identify ducts with BP1 expression (red color indicates BP1 staining). The ellipse defines BP1 negative cells within a BP1 positive duct. 200×. (E)–(F) Ductal carcinoma *in situ* (DCIS). Expression of BP1 in DCIS is heterogeneous. (E) Cells in one duct are homogeneously immunoreactive, while the other two ducts contain many BP1 negative cells (arrow). (F) Some of the *in situ* and hyperplastic lesions are strongly positive for BP1 (dark arrows), while the others are weakly positive or negative for BP1 (light arrow) 150×. (G)–(H) Invasive ductal carcinoma. The infiltrating cancer cells are strongly BP1 positive (red color), while the trapped normal ducts are negative (arrows) 150×.

Table 2. Numbers of BP1 (+) ducts and foci among normal and different breast lesions

Lesion	Number of ducts and foci	BP1 positive (%)
Normal	193	17 (8.8%)
Hyperplasia	374	67 (17.9%)
In situ	325	101 (31.1%)
Invasive	121	95 (78.5%)

Histological type differences significant at $p < 0.0001$.

ducts were frequently distributed as clusters with a defined boundary separating adjacent BP1 negative ducts (Figure (1B)–(D)) indicating heterogeneity of expression. It is interesting to note that over 90% of the benign intraductal papillomas (a subset of the hyperplasia samples) were BP1 negative. In the *in situ* tumors, 325 ducts were assessed; 101 (31%) were BP1 positive. The expression of BP1 in the *in situ* tumors was also heterogeneous, with positive and negative cells co-existing in different sites of the same duct (Figure (1E)–(F)). Of 121 invasive foci analyzed, 95 (78.5%) were BP1 positive. The cells in invasive lesions were generally uniformly BP1 positive (Figure (1G)–(H)). The frequency of BP1 positivity in 82 cases with co-existing normal, hyperplasia, *in situ*, and invasive components was 18.3, 32.9, 47.5, and 82.9%, respectively ($p < 0.0001$) (Table 3; Figure (2A)–(B)).

In the cases examined above, distinct BP1 immunoreactivity was preferentially present in epithelial cells. A majority of the stromal elements, including fibroblasts, leukocytes, and smooth muscle cells were devoid of BP1 expression. BP1 was predominantly distributed in the cytoplasm, while it was also occasionally seen in the nucleus of a subset of normal, benign, and malignant cells (not shown).

Table 3. BP1 expression in 82 cases with co-existing normal and different breast lesions

Lesion	BP1 positive (%)
Normal	15 (18.3%)
Hyperplasia	27 (32.9%)
In situ	38 (46.3%)
Invasive	68 (82.9%)

Tissue component differences significant at $p < 0.0001$.

Table 4. Cell proliferation rates in morphologically similar BP1 (+) and (−) cells

Cell type	Cells counted	Ki-67 positive (%)
BP1 positive	5000	360 (7.2%)
BP1 negative	5000	170 (3.4%)

Cell type differences significant at $p < 0.0001$.

Co-localization of BP1 and erbB2

Of 15 previously identified erbB2 positive tumors, BP1 was co-localized with erbB2 in a subset of the tumor cells in each of the cases. The number of cells with co-localized BP1 and erbB2 increased with the progression from an *in situ* to invasive status (Figure (2C)–(F)). BP1 positive cells, however, were far greater in number than erbB2 cells.

BP1 expression is associated with proliferation

To monitor BP1 expression associated with cell proliferation, sections from 20 morphologically similar *in situ* and invasive tumors with ($n = 10$) and without ($n = 10$) BP1 expression were double immunostained for Ki-67 and BP1. In addition, immunostaining of Ki-67 and BP1 was performed on two adjacent sections. Ductal cells with BP1 expression had a significantly higher rate of cell proliferation, 7.2 versus 3.4% ($p < 0.0001$) (Table 4; Figure (2G)–(H)). BP1 immunostaining was cytoplasmic, in contrast to the nuclear staining of Ki-67.

Discussion

Our current study reveals that the distribution and intensity of BP1 expression increase with the extent of cellular proliferation and carcinogenesis (normal→ hyperplasia→ *in situ*→ invasive), from a few randomly distributed BP1 positive cell clusters in normal controls to distinct immunoreactivity in the vast majority of cells in 81% of the invasive tumors. Compared to normal, hyperplastic, and *in situ* lesions, invasive lesions consistently showed the highest number of BP1 positive cells, and the highest intensity of BP1 immunoreactivity. This was even more apparent in cases with co-existing normal, hyperplastic, *in situ*, and invasive components (Figure (2A)–(B)).

Double immunostaining showed that BP1 is co-localized with erbB2 in a subset of the tumor cells in each of 15 erbB2 positive tumors, and that the number of cells with co-localized BP1 and erbB2 increases with the progression from an *in situ* to invasive status. BP1 maps near erbB2 [12], and we speculate these two genes may be co-amplified. Future studies will examine whether there is a direct relationship between expression of BP1 and erbB2.

BP1 protein is preferentially located in the cytoplasm. Although translation occurs in the cytoplasm, the increasingly intense BP1 staining that was observed with tumor progression, coupled with the lack of nuclear staining, may suggest a functional role for BP1 in the cytoplasm. There are a growing number of transcription factors that localize to the cytoplasm. A HOX co-factor protein, PREP-1, is localized in the nucleus in murine primary oocytes but in the cytoplasm of mature oocytes; the functional significance of this is unknown [18]. Our previous studies revealed that a putative transcription
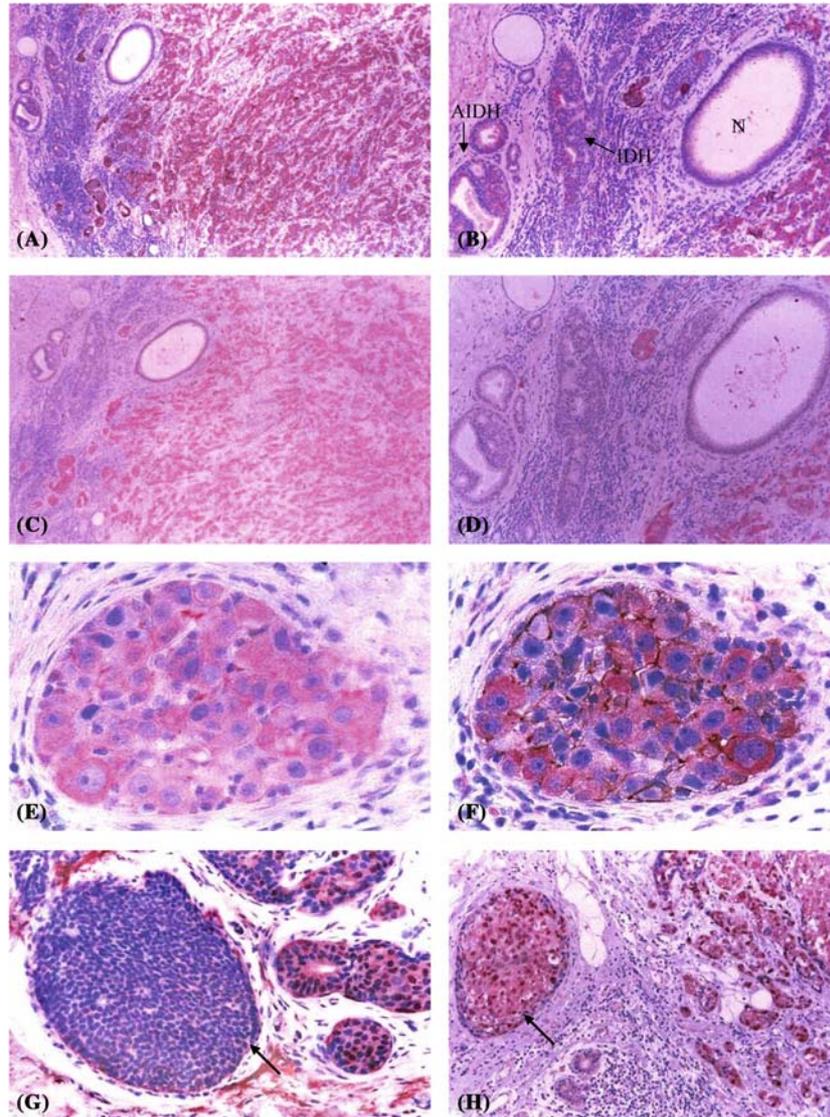
Figure 2. Correlation of BP1 expression with erbB2 and cell proliferation status. (A) A section containing multiple components was immuno-stained for BP1. 40×. (B) Higher magnification of (A). 100×. The infiltrating cancer cells are uniformly positive for BP1, while the normal (N), typical intraductal hyperplasia (IDH), and atypical intraductal hyperplasia (AIDH) are either negative or weakly positive. (C) The section adjacent to (A), immunostained for erbB2. Note that the normal cells, IDH, and AIDH are completely negative for erbB2, while the infiltrating cancer cells are uniformly erbB2 positive 40×. (D) A higher magnification of C 100×. (E) Section of DCIS immunostained for BP1 400×. (F) The section adjacent to E, double immunostained for BP1 (red) and erbB2 (brown). Note that BP1 and erbB2 are co-localized some cells 400×. (G)–(H) Sections of DCIS and IDC, double immunostained for BP1 + smooth muscle actin (red) and Ki-67 (brown). (G) DCIS without distinct BP1 immunostaining (arrow) has few Ki-67 positive cells, while a vast majority of adjacent BP1 positive cells are Ki-67 positive. (H) A separate case of DCIS with distinct BP1 expression contained substantially more Ki-67 positive cells.

factor, heterogeneous nuclear ribonucleoprotein (hnRNP) A2/B1, is exclusively or preferentially located in the nucleus of normal respiratory cells, while it becomes increasingly detectable in the cytoplasm of hyperplastic and neoplastic cells during tumor progression. Cells with cytoplasmic hnRNP A2/B1 immunore-activity had a 3-fold higher frequency of microsatellite alterations and loss of heterozygosity than did cells with nuclear hnRNP A2/B1 immunoreactivity [19]. Some homeotic proteins can be transported to the cytoplasm and secreted, then taken up by other cells [20]. Other transcription factors demonstrate phosphorylation-dependent and ligand-dependent transport from cyto-plasm to nucleus, such as occurs with Smad and ER proteins respectively [21–23]. Cytoplasmic-nuclear shut-tling would be a new property of BP1 protein, one with important implications if true; future studies will investi-gate whether this occurs and the underlying mechanism.

The tissue samples used in our current study are from various states in the United States and from other countries, including Japan and China, which have been reported to have a significantly lower breast cancer incidence than Western countries. The frequency and pattern of BP1 expression among different ethnic groups and people from different areas, however, are very sim-ilar, suggesting that BP1 might be a highly conserved protein and an important participant in a major common pathway of breast tumor progression. A major disad-

vantage of the diversity in our tissue sample resources is that it is difficult or impossible to follow-up the patients and to assess clinical correlations. Currently, we are assessing the clinical significance of BP1 expression and correlation between BP1 and other molecules, using the samples from the George Washington University Medical Center, which has detailed clinical information. Our preliminary immunohistochemical findings are consistent with those of our current study.

Our current and past findings suggest that BP1 may be an important upstream factor in an oncogenic pathway. While the exact role(s) of BP1 in breast tumor development and progression has not been defined, BP1 appears to be specifically involved in blocking apoptosis and facilitating the formation and expansion of a biologically more aggressive cell clone, since: (1) In our previous studies BP1 is aberrantly expressed in 63% of the bone marrows of acute myeloid leukemia patients, including 81% of pediatric and 47% of adult patients, as well as in 32% of pediatric T-cell acute lymphocytic leukemia patients. In contrast, BP1 mRNA is barely if at all detectable in normal BM and PHA-stimulated T cells [7]; (2) Ectopic expression of BP1 in the leukemia cell line K562 substantially increased their clonogenicity [7], suggesting that BP1 is capable of sustaining or facilitating deregulated cell proliferation, which has been regarded as a direct cause of malignancy [24, 25]; (3) Abrogation of BP1 expression in K562 cells causes apoptosis (P.E.B., unpublished data). (4) BP1 mRNA was detectable in 80% of breast IDCs, whereas it was detectable in only one of nine matched normal controls as measured by RT-PCR ([2]; unpublished data), and BP1 expression was significantly higher in ER negative than in ER positive breast tumors, and in the tumors of African Americans than in Caucasians [8]; (5) Breast cancer cells expressing BP1 have a significantly higher cell proliferation index, compared to their morphologically comparable counterparts without BP1 expression (see Figure (2G)–(H)); (6) BP1 positive cells are generally distributed as distinct clusters or foci with a well-defined boundary to adjacent BP1 negative cell clusters (see Figure (1B)–(D)), consistent with the typical feature of clonal proliferation and expansion [26]; (7) In preliminary studies, injection of MCF7 cells that overexpress BP1 into the fat pads of nude mice caused increased numbers of breast tumors compared with controls (P.E.B. and B. Vonderhaar, unpublished data).

Conclusions

Our findings reveal that the frequency, distribution and intensity of BP1 expression increase with tumor progression. Taken together, our results suggest that BP1 is an important upstream factor in an oncogenic pathway(s) and may directly contribute to tumor progression and/or invasion.

References

1. Chen H, Sukumar S: Role of homeobox genes in normal mammary gland development and breast tumorigenesis. J Mammary Gland Biol, Neoplasia 8: 159–175, 2003
2. Cantile M, Pettinato G, Procino A, Feliciello I, Cindolo L Cillo C: *In vivo* expression of the whole HOX gene network in human breast cancer. Eur J Cancer 39: 257–264, 2003
3. Care A, Silvani A, Meccia E, Mattia G, Peschle C, Colombo MP: Transduction of the SkBr3 breast carcinoma cell line with the HOXB7 gene induces bFGF expression, increases cell proliferation and reduces growth factor dependence. Oncogene 16: 3285–3289, 1998
4. Care A, Felicitti F, Meccia E, Bottero L, Parenza M, Stoppacciaro A, Peschle C, Colombo MP: HOXB7: a key factor for tumor-associated angiogenic switch. Cancer Res 61: 6532–6539, 2001
5. Raman V, Martensen SA, Reisman D, Evron E, Odenwald WF, Jaffee E, Marks J, Sukumar S: Compromised HOXA5 function can limit p53 expression in human breast tumors. Nature 405: 974–978, 2000
6. Chase MB, Fu S, Haga SB, Davenport G, Stevenson H, Do K, Morgan D, Mah AL, Berg PE: BP1, a homeodomain-containing isoform of DLX4, represses the beta-globin gene. Mol Cell Biol 22: 2505–2514, 2002
7. Haga SB, Fu S, Karp JE, Ross DD, Williams DM, Hankins WD, Behm F, Ruscetti FW, Chang M, Smith BD, Becton D, Raimondi SC, Berg PE: BP1, a new homeobox gene, is frequently expressed in acute leukemias. Leukemia 14: 1867–1875, 2000
8. Fu SW, Schwartz A, Stevenson H, Pinzone JJ, Davenport GJ, Orenstein JM, Gutierrez P, Simmens SJ, Abraham J, Poola I, Stephan DA, Berg PE: Correlation of expression of BP1, a homeobox gene, with estrogen receptor status in breast cancer. Breast Cancer Res 5: 82–87, 2003
9. Schmitt FC: Multistep progression from an oestrogen-dependent growth towards an autonomous growth in breast carcinogenesis. Eur J Cancer 31A: 2049–2052, 1995
10. Sheikh MS, Garcia M, Pujol P, Fontana JA, Rochefort H: Why are estrogen receptor negative breast cancers more aggressive than the estrogen receptor positive breast cancers? Invas Metast 14: 329–336, 1994–95
11. Kaptain S, Tan LK, Chen B: Her-2/neu and breast cancer. Diagn Mol Pathol 10: 139–152, 2001
12. Fu S, Stevenson H, Strovel JW, Haga SB, Stamberg J, Do K, Berg PE: Distinct functions of two isoforms of a homeobox gene, BP1 and DLX7, in the regulation of the beta-globin gene. Gene 278: 131–139, 2001
13. Tavassoli FA, Man YG: Morphofunctional features of intraductal hyperplasia, atypical hyperplasia, and various grades of intraductal carcinoma. Breast J 1: 155–162, 1995
14. Man YG, Ball WD, Culp AJ, Hand AR, Moreira JE: Persistence of a perinatal cellular phenotype in the ducts of adult glands. J Histochem Cytochem 43(12): 1203–1215, 1995
15. Man YG, Tavassoli FA: A simple epitope retrieval method without the use of microwave oven or enzyme digestion. Appl Immunohistochem 4(2): 139–141, 1996
16. Tsuda H, Sasano H, Akiyama F, Kurosumi M, Hasegawa T, Osamura RY, Sakamoto G: Evaluation of interobserver agreement in scoring immunohistochemical results of HER-2/neu (c-erb B-2) expression detected by Hercep Test, Nichirei Polyclonal antibody

CB11 and TAB250 in breast carcinoma. Pathol Int 52: 126–134, 2002

17. Birner P, Oberhuber G, Stani J, Reithofer C, Samonigg H, Hausmaninger H, Kubista E, Kwasny W, Kandioler-Eckersberger D, Gnant M, Jakesz R, and the Austrian Breast & Colorectal Cancer Study Group: Evaluation of the United States Food and Drug Administration approved scoring and test system of HER-2 protein expression in breast cancer. Clin Cancer Res 7: 1669–1675, 2001

18. Villaescusa JC, Verrotti AC, Ferretti E, Farookhi R, Blasi F: Expression of Hox cofactor genes during mouse ovarian follicular development and oocyte maturation. Gene 330: 1–7, 2004

19. Man YG, Martinez A, Avis IM, Hong SH, Cuttitta F, Venzon DJ, Mulshine JL: Phenotypically different cells with heterogeneous nuclear ribonucleoprotein A2/B1 overexpression show similar genetic alterations. Am J Resp Cell Mol Biol 23: 636–645, 2000

20. Prochiantz A, Joliot A: Can transcription factors function as cell–cell signaling molecules? Nat Rev Mol Cell Biol 4: 814–819, 2003

21. Derynck R, Zhang YE: Smad-dependent and Smad-independent pathways in TGF-β family signaling. Nature 425: 577–584, 2003

22. Pinzone JJ, Stevenson H, Strobl JS, Berg PE: Molecular and cellular determinants of estrogen receptor-α expression. Mol Cell Biol 24: 4605–4612, 2004

23. Murphy L, Cherlet T, Lewis A, Banu Y, Watson P: New insights into estrogen receptor function in human breast cancer. Ann Med 35: 614–631, 2003

24. Pan H, Yin C, Van Dyke T: Apoptosis and cancer mechanism. Cancer Surv 29: 305–327, 1997

25. Pierce GB, Speers WC: Tumors as caricatures of the process of tissue renewal: prospects for therapy by directing differentiation. Cancer Res 48: 1990–2004, 1996

26. Man YG, Ball WD, Marchetti L, Hand AR: Contributions of intercalated duct cells to normal parenchyma of submandibular glands of adult rats. Anat Rec 263: 202–214, 2001

Address for offprints and correspondence: Dr Patricia E. Berg, PhD, Department of Biochemistry and Molecular Biology, George Washington University Medical Center, 2300 Eye Street, NW, Washington DC 20037, USA; *Tel.:* +1-202-994-2810; *Fax:* +1-202-994-8974; *E-mail:* bcmpeb@gwumc.edu